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14. ABSTRACT

We hypothesize that GR mediates resistance to enzalutamide in advanced prostate cancer through transactivation of SGK1. In this proposal, we aim to further explore the relationship between GR, SGK1, and enzalutamide resistance through experimental manipulation of GR and SGK1 in our previously reported laboratory models of enzalutamide resistance. In this research period we have further expanded upon the relationship between GR and SGK1 in the context of enzalutamide-driven prostate cancer. We have generated CRISPR/Cas9 cell lines to test the importance of SGK1. Finally, we have used the recently published metastatic prostate cancer genomics dataset to identify a novel mechanism of enzalutamide resistance that correlates with the effects of SGK1 in our xenograft models. This project has the potential to identify a critical and druggable component of the GR program to enable a safer and more effective strategy for treating AR-independent enzalutamide-resistant prostate cancers.

15. SUBJECT TERMS

Glucocorticoid Receptor, Serum and glucocorticoid-regulated kinase 1, SGK1, epithelial-mesenchymal transition, EMT

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INTRODUCTION

Castration-resistant prostate cancer (CRPC) inevitably develops resistance to the 2nd generation androgen receptor (AR) antagonist, enzalutamide, despite continued enzalutamide-mediated suppression of AR activity. A recent study from the Sawyers laboratory found that the nuclear hormone receptor transcription factor, glucocorticoid receptor (GR), can activate an overlapping set of AR target genes and can mediate resistance to enzalutamide in a mouse xenograft model. This study and others have led to considerable efforts to develop GR antagonists for the treatment of GR-driven prostate cancer. However, pharmacologic inhibition of GR-dependent physiology is of unknown clinical feasibility and we proposed to test whether serum and glucocorticoid-regulated kinase 1 (SGK1), a target gene of the GR transcriptional program, might be more suitable for targeted inhibition. GR and serum and glucocorticoid-regulated kinase 1 (SGK1) were among the most upregulated genes in an *vivo* model of enzalutamide resistance. We hypothesize that GR mediates resistance to enzalutamide through transactivation of SGK1. In this proposal, we aim to further explore the relationship between GR, SGK1, and enzalutamide resistance through experimental manipulation of GR and SGK1 in our previously reported laboratory models of enzalutamide resistance. This project has the potential to identify a critical and druggable component of the GR program to enable a safer and more effective strategy for treating AR-independent enzalutamide-resistant prostate cancers.

KEYWORDS

Androgen Receptor, AR
Glucocorticoid Receptor, GR
Serum and Glucocorticoid-Regulated Protein Kinase 1, SGK1
Epithelial-Mesenchymal Transition, EMT
Prostate Cancer
Castration-Resistant Prostate Cancer, CRPC

PROJECT GOALS

1. Validate SGK1 as a driver of GR-dependent resistance to enzalutamide
2. Explore the role of SGK1-mediated epithelial-mesenchymal transition in CRPC

Phase 1 Milestones (proposed completion September 2016)

1. Modulate SGK1 expression in a previously established GR-driven prostate cancer cell line model and determine the effects on resistance to enzalutamide and AR activity
2. Determine the importance of SGK1-dependent cellular reprogramming in a 3D organoid human prostate cancer culture system.

Task	% Completed	Status
Clone SGK1 overexpression construct	100%	Complete
Generate SGK1 overexpressing cell models	50%	Ongoing
Clone SGK1 CRISPR	100%	Complete
Generate SGK1-deficient cell models	75%	Ongoing
Test SGK1 inhibitor	100%	Complete

Treating SGK1-deficient cells with enzalutamide	50%	Ongoing

Phase 2 Milestones (proposed completion September 2017)
Evaluate the importance of EMT-related cellular reprogramming in SGK1-dependent enzalutamide resistance

Task	**% Completed**	**Status**
Develop flow cytometry-based EMT assay	25%	Ongoing
Generate loss of function EMT models	25%	Ongoing
Generate gain of function EMT models	25%	Ongoing

ACCOMPLISHMENTS

In our preliminary data we reported that the GR target gene SGK1 could confer resistance to enzalutamide in a xenograft model of advanced prostate cancer. We further showed that RNA analysis of the SGK1-overexpressing tumors showed evidence that SGK1 could mediate epithelial-mesenchymal transition and downregulation of RNA sequencing analysis of a panel of 7 prostate cancer organoid cultures recently derived identified a dramatic concordance of SGK1 and EMT-related gene expression (Figure 1). Importantly, this upregulation of SGK1 and EMT program inversely correlated with AR expression. SK-PCa1 and SK-PCa2 were chosen as representative cell lines for further analysis. Qualitatively, PCa1 demonstrates a strong EMT phenotype ($CDH1^{low}$, $CDH2^{high}$) as well as relatively high SGK1, and undetectable AR. In distinction, PCa2 demonstrates weak evidence of EMT ($CDH1^{high}$, $CDH2^{low}$), relatively low SGK1, and high AR (data not shown). This 3D organoid system will be used to further test the importance of SGK1 in coordinating EMT.

Collectively, our data led us to hypothesize that the glucocorticoid receptor (GR) can promote resistance to enzalutamide, in part, through upregulation of its target gene, SGK1.

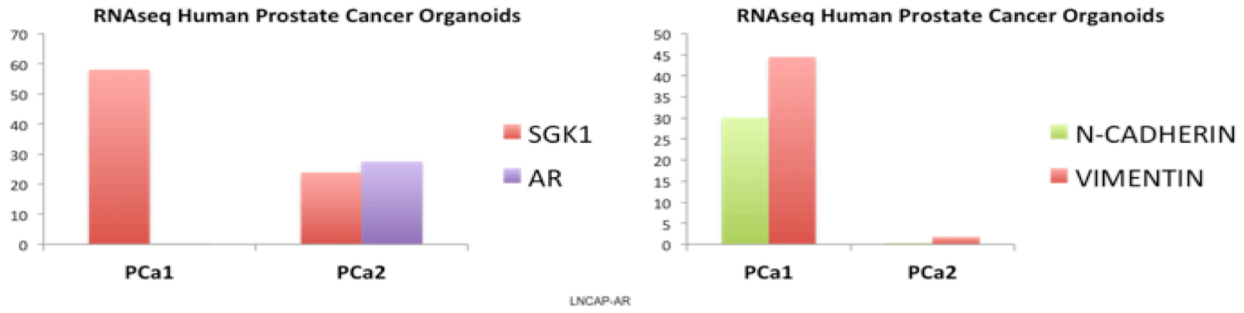


Figure 1. Effect of SGK1 overexpression on enzalutamide resistance and EMT gene expression
A. LNCAP-AR cells expressing either GFP, SGK1 (wild-type), SGK1 (kinase-dead) were injected into enzalutamide treated mice. Tumor volumes shown are the average +/- standard error. Western blot of the above cell lines shows SGK1 and actin protein levels. B. mRNA collected from SGK1 (wild-type) and SGK1 (kinase-dead) was analyzed via Illumina array. Fold differences in mRNA expression is depicted as the average of 4 SGK1 (wild-type) and 2 SGK1-kinase dead control.

SGK1 and GR-dependent advanced prostate cancer

To further interrogate the importance of SGK1 in mediating GR-driven enzalutamide resistance, GR-expressing enzalutamide-resistant prostate cancer cells expressing CRISPR/Cas9 and a guide targeting SGK1 (sgSGK1) were generated. sgSGK-1 expressing cells showed a moderate reduction in the phosphorylation of NDRG1 at T346, which is a previously reported target of SGK1 kinase activity (Figure 2). In vitro and in vivo studies testing the importance of SGK1 in GR-dependent enzalutamide resistance are ongoing.

Our study was predicated on the finding that SGK1 is an important transcriptional target of GR and that strategies that might therapeutically interfere with SGK1 activity might enable interference with GR-driven resistance to enzalutamide. To confirm that GR is in fact relevant in human prostate cancer, we leveraged the recently derived human prostate cancer organoids (1). These organoids lines were all derived from fresh metastatic biopsies from patients who had all developed castration-resistant advanced prostate cancer. Western blotting of protein lysates extracted from these organoid lines revealed detectable GR protein expression in six out of the seven lines (86%) (Figure 3). Interestingly, there appeared to be two distinct molecular weight GR species present, and previous literature has indeed detected multiple GR isoforms with significantly different activities (Figure 3) (2). Given the isoform-specific GR expression that we detected in multiple prostate cancer specimens and our hypothesis that GR drives enzalutamide resistance through activation of SGK1, it became absolutely critical for us determine whether all of the major GR isoforms can activate SGK1.

Figure 2. CRISPR/Cas9-mediated knockdown of SGK1 in the enzalutamide-resistant LNCAP-AR derived cell line, LREX'. Western blotting of extracts derived from LREX' infected with lentivirus expressing CRISPR sgRNA targeting GFP (control) and CRISPR sgRNA targeting SGK1 is shown.

Figure 3. GR is expressed in the majority of advanced CRPC. Western blotting of prostate cancer organoid lines grown in ADMEM/F12 with defined growth factors on collagen coated plates.

GR isoforms have received significant attention in the literature but have not been described in advanced prostate cancer. The dominant GR mRNA can be alternatively translated from one of 8 different translational start sites, labeled A, B, C1, C2, C3, D1, D2, and D3 (Figure 4). In vitro reporter assays demonstrate that GRα-C3 isoform is a more potent driver of glucocorticoid responsive genes than the full length GRα-A, whereas the D family of GRα isoforms has a significantly weaker ability to activate target genes (2). Given the vastly different transcriptional outputs of the GR isoforms, our previous finding that GR is a driver of enzalutamide resistance (3) raised the question as to which isoform of GR is the principal driver of prostate cancer pathogenesis and which of these isoforms can lead to upregulation of SGK1.

To formally study the functional significance of these isoforms as drivers of SGK1 and enzalutamide resistance, we returned to our most robust model of GR-driven enzalutamide resistance, the LNCAP-AR derived human prostate cancer cell line, LREX. In fact LREX cells also possess 2 distinct isoforms of GR (Figure 5) providing us with a relevant model to test the contributions that each GR variant makes towards SGK1 upregulation. To investigate which isoforms of GR can drive resistance to enzalutamide in this model, we used the CRISPR/Cas9 system in a novel way to generate isoform-specific frameshift mutations (Figure 5). The

CRISPR/Cas9 system enables insertion of insertions and/or deletion into specific genomic DNA sequences. Using this system, guide RNAs were designed to insert genomic alterations in the genomic DNA downstream of the GR full-length translational start site at codon 1 in order to create a frameshift mutation. Because the truncated GR-C isoform is translated from codon 98, we hypothesized that any insertion or deletion upstream of this codon would not interfere with its translational initiation. Immunoblotting and Sanger sequencing confirmed that the sgGRA guide deleted GRα-A while sparing GRα-C (Figure 5).

Consistent with the capability of GR-C to drive enzalutamide resistance, LREX cells with lentivirus expressing sgGRA formed significantly larger xenograft tumors in enzalutamide-

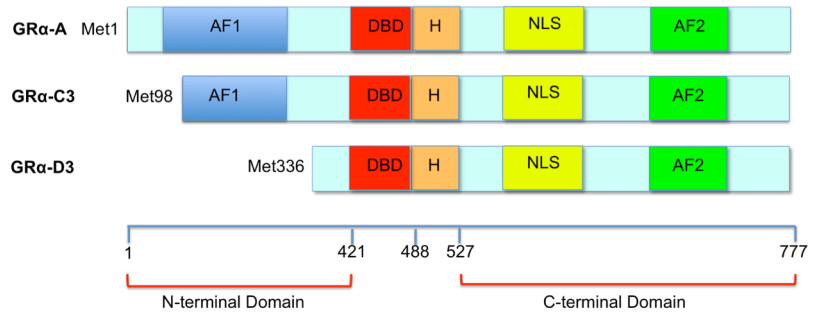


Figure 4. The translational isoforms of GR. GRα-A, the canonical full-length isoform of GR is comprised of an N-terminal activation function domain (AF1), a DNA-binding domain (DBD), a hinge region (H), a nuclear localization signal (NLS), and a C-terminal activation function domain (AF2). The N-terminal truncation variant GRα-C, distinguished as the isoform with the most potent in vitro transcriptional activity, lacks the first 97 amino acids and possesses a truncated AF1 domain.

treated mice then cells infected with control sgRNA (sgCTRL) (610 mm^3 vs. 396 mm^3, $p < 0.05$) (Figure 5). These data demonstrate GRα-C is sufficient to drive the progression of enzalutamide-resistant prostate cancer and is possibly able to do so more potently than GRα-A. Once we had demonstrated that GR-A and GR-C could both drive enzalutamide resistance we next turned to the link between GR isoforms and SGK1.

To determine whether SGK1 can be upregulated by GR-C as well as GR-A, protein lysates were extracted from GR-A/C expressing tumors as well as from GR-A expressing tumors. Strikingly, SGK1 expression remained constant in both sets of tumors (Figure 6) suggesting to us that GR-C might be sufficiently capable to drive SGK1 expression. This also raised the hypothesis that SGK1 activation by GR is a critical component of GR-driven enzalutamide resistance. To formally test whether GR-A and GR-C can both transcriptionally activate SGK1, we expanded an LREX' clonally derived cell line which showed no expression of GR on immunoblot (LREX GR-null) and we obtained cDNAs of GR with point mutations in the GR-A, C, and GR-D start sites so as to enable isoform-specific expression. LREX GR-null cells were infected with a lentivirus either expressing empty vector or expressing a doxycycline inducible GR-A or GR-C cDNA. Cells where then treated with doxycycline 250ng/ml and dexamethasone 100nM for 24hr and protein lysates were made. SGK1 levels were found to be equivalent in cells expressing GR-A and GR-C and cells expressing each of these isoforms individually (Figure 7). The effects of doxycycline itself on activating SGK1 itself were minimal (data not shown). Collectively, this experiment provides further support to the hypothesis that the dominant translational isoforms of GR, GR-A and GR-C, can both transcriptionally activate SGK1.

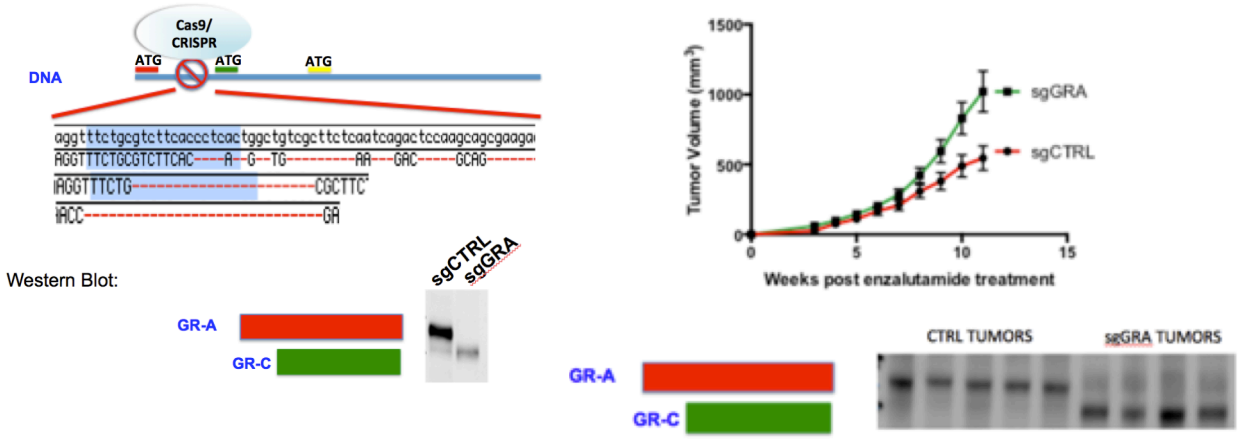


Figure 5. **CRISPR/Cas9-mediated isoform-specific deletion of GR-C.** Top Panel. Schematic of the GR locus and sanger sequencing of three independent clones confirming homozygous frameshift deletions. Bottom Panel. Schematic of the translational isoforms, GR-A and GR-C and immunoblot of lysates with a c-terminal GR antibody. GR-C can drive enzalutamide-resistant prostate cancer. Top Panel. Xenograft growth curves implanted in castrated NOD/SCID mice treated with enzalutamide 10mg/kg from the day of implantation.

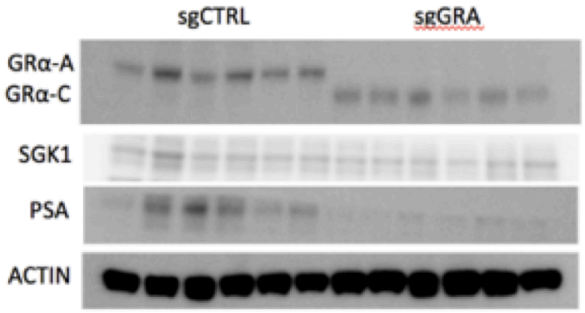


Figure 6. SGK1 is expressed in prostate xenograft tumors expressing either GR-A or GR-C. Immunoblot of protein extracts from xenografts. Antibodies against GR, SGK1, PSA, Actin were used.

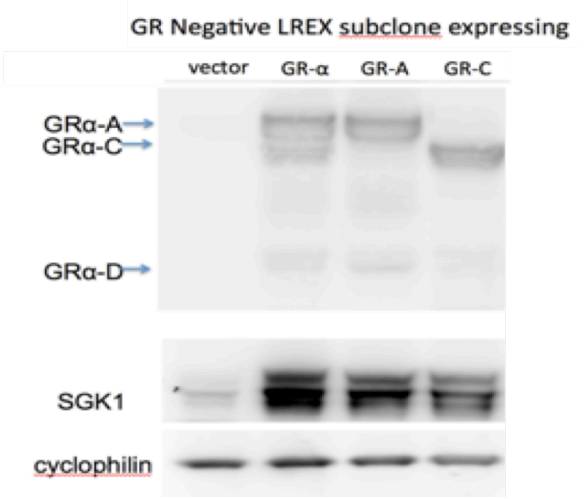


Figure 7. GR-A and GR-C activation leads to the upregulation of SGK1. Anti-GR immunoblot of lysates extracted from LREX GR-null cells expressing either GR-A and GR-C or the individually expressing either GR-A or GR-C treated with doxycycline 250ng/ml

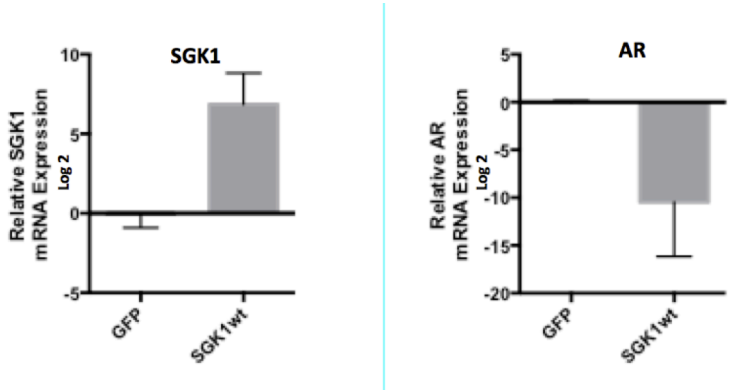
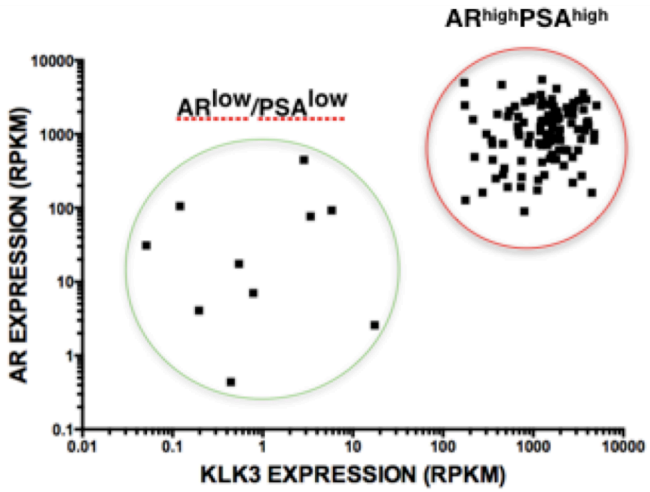


Figure 9. SGK1 overexpression can lead to downregulation of AR. RNA was extracted from human LNCAP-AR derived xenografts constitutively overexpressing SGK1 (SGK1wt) or GFP control.

These data demonstrate for the first time that advanced prostate cancers can express translational isoforms of GR in a selective fashion and that SGK1 can be upregulated by the two dominant GR isoforms, GR-A and GR-C. Our next step will be further validate the functional significance of SGK1 in GR isoform-specific cell lines. To carry these experiments out we will use commercially available chemical inhibitors of SGK1 as well as CRISPR/Cas9 based silencing of SGK1. This approach will be used to test our hypothesis in both prostate organoids as well as human cell line models in a xenograft model.

SGK1, EMT, and AR negative prostate cancer

Consistent with the role of SGK1 in mediating EMT-like changes and correlating with loss of AR expression, the gene expression analysis of SGK1-expressing tumors showed evidence of a decrease in AR expression (Figure 9). Our finding that SGK1 is associated with features of lineage transdifferentiation including loss of AR expression and upregulation of mesenchymal markers prompted us to further explore the association of AR activity and mesenchymal marker expression in currently available prostate cancer genomics datasets. This was also important to us because we wanted to assure ourselves that loss of AR expression is a clinically prevalent and therefore meaningful event in the setting of CRPC. In particular, loss of AR expression is significant in that it reflects an obvious resistance mechanism to AR-directed therapies. To determine whether AR expression is lost at a measurable rate in CRPC, we analyzed the recently published genomics dataset that including metastatic biopsies from 150 patients with castration-resistant prostate cancer (4). Analysis of AR and PSA expression in this dataset identified a cohort of patients with virtually absent AR and PSA expression (Figure 10). Importantly, the fractional tumor content of the AR^{low}/PSA^{low} was equivalent to the $AR^{high}PSA^{high}$ tumors ruling out the possibility of stromal contamination diluting out the AR and PSA mRNA.



	$AR^{low}PSA^{low}$	$AR^{high}PSA^{high}$	P value
Fraction Tumor content	0.66	0.60	0.39

Figure 10. Castration-resistant prostate cancer can be divided into clusters by AR and PSA expression. Top Panel. AR and PSA (KLK3) mRNA levels in 150 cases measured by next-generation RNA sequencing. Bottom Panel. Fraction tumor content determined by histopathologic assessment.

Importantly, the AR^{low} /PSA^{low} cases were enriched in genomic alterations in known tumor suppressors implicated in lineage transdifferentiation. 50% of AR low cases possessed either Rb1 deletion or nonsense mutation compared to only 4% of the remainder of the cohort ($p< 0.0001$). These genes have been previously linked with lineage transdifferentiation and aggressiveness in multiple different malignancies (5-7). This data suggests to us that loss of AR represents a significant and prevalent finding in advanced prostate cancer and that our laboratory discovery that SGK1 might contribute to loss of AR has relevance in clinic. Consistent with the relationship between GR, AR, and SGK1 that we have observed in our laboratory models, we found an inverse relationship between SGK1 mRNA and AR mRNA levels across all cases of castration-resistant prostate cancer (Pearson coefficient = -0.2) whereas we found a positive correlation between GR and SGK1 (Pearson coefficient = 0.4). Furthermore gene expression analysis of these AR negative cases relative AR positive cases allowed us to derive a signature of genes for novel biological pathway discovery. Importantly, as with our SGK1 overexpression model we found multiple genes involved in lineage fate determination, including genes involved in epithelial-mesenchymal transition.

This analysis will allow us to complete our next step of experiments as outlined in year 2 of the statement of work for this grant proposal which will be to investigate the role of epithelial-mesenchymal transition in mediating resistance to AR-directed therapies. These experiments will be carried out in our SGK1-overexpression model as well as in the physiologically relevant organoid models of which several have loss of AR and upregulation of alternative lineage markers including epithelial-mesenchymal transition.

Pending Goals

Overexpression of SGK1 in SK-PCa2 and silencing of SGK1 in SK-PCa1 prostate organoid models is ongoing.

Training Opportunities

This year I had the opportunity to attend the annual American Society of Clinical Oncology conference at which I attended multiple workshops geared towards translational oncologists. Topics discussed include advanced workshops in management of prostate cancer patients, molecular heterogeneity in cancer, using cell-free DNA to guide oncologic management, etc.

To facilitate progress on this project, I had the opportunity to train a new laboratory technician. I trained this technician to become proficient in in vitro tissue culture, PCR, quantitative PCR, immunoblotting, sequencing analysis, and CRISPR/Cas9 gene editing.

To facilitate my clinical-translational skills, I took part in a statistics seminar course that covered topics in descriptive and inferential statistics including hypothesis testing, regression, and multivariate analysis. I look forward to continuing my statistics and data science training with further coursework this year offered at MSKCC and through massive open online courses (MOOCs).

Dissemination to communities of interest: Nothing to report

PLANS FOR PROPOSAL YEAR 2

FUNCTIONAL STUDIES OF SGK1

Based on our finding that both GR-A and GR-C can lead to upregulation of SGK1, we will also test the effect of silencing SGK1 on enzalutamide resistance in both GR-A and GR-C-expressing enzalutamide-resistant cells. Silencing and overexpressing SGK1 in SK-PCa1 and SK-PCa2 organoids as well as overexpressing SGK1 in PCa1 and PCa2 will be accomplished using shRNA, CRISPR/Cas9, and chemical inhibitors. In vitro resistance to enzalutamide will be assessed using commercially available cell viability assays and through our in vivo enzalutamide-resistance xenograft assay.

STUDIES ON EMT AND CRPC

If SGK1 is found to be critical for enzalutamide resistance in our human cell line and organoid models then we will modulate the EMT program in enzalutamide-resistant cells LREX and SK-PCa1 to determine if EMT is important in SGK1-driven enzalutamide resistance. We will modulate known EMT inducers that we can directly link to SGK1 activity. Candidate EMT inducers that will need to be validated as SGK1-driven, include TWIST1, the EMT inducer found to be overexpressed in SK-PCa1 by RNA sequencing. We will use a CDH1/CDH2 immunofluorescence-based assay coupled with flow cytometry to measure the effects of our modified cell lines on EMT output. In vitro resistance to enzalutamide will be assessed using commercially available cell viability assays and through our in vivo enzalutamide-resistance xenograft assay.

IMPACT

CURRENT DISCIPLINE

1. GR variants, SGK1, and Prostate Cancer

Our discovery that translational isoforms of GR can drive resistance to enzalutamide in advanced prostate cancer will impact our ability both to detect and treat advanced prostate cancer. Because GR-C lacks the N-terminal domain present on GR-A, it cannot be detected by N-terminal GR-directed antibodies. This finding provides specific guidance on the type of GR-directed antibody that needs to be used to detect GR upregulation in advanced prostate cancer specimens.

Our preliminary data and the work of others suggest that GR-C might be more active than GR-A. This could further shed light on the aggressiveness of GR-C dependent prostate cancer recurrence. Furthermore, because of the known interaction between the N-terminal domain and C-terminal domain in mediating ligand-dependent GR activation, we hypothesize that the truncated GR variant, GR-C might respond differentially to GR antagonists that are being developed for use in the treatment of advanced prostate cancer.

Finally, our finding that GR-A and GR-C activity can lead to upregulation of SGK1 continues to strengthen the connection between GR, SGK1, and prostate cancer. The fact that GR-A and GR-C both upregulate SGK1 and promote resistance to AR-directed therapies suggests the hypothesis that SGK1 is a critical component of the GR output that facilitates resistance to

enzalutamide. SGK1 is a druggable kinase and its active presence in prostate cancer pathogenesis suggests that targeted therapeutic inhibition of SGK1 may be an effective strategy in a large proportion of patients with advanced prostate cancer.

2. Loss of AR in advanced prostate cancer

Our finding that SGK1 overexpression leads to downregulation of AR in a laboratory model of prostate cancer led us to interrogate the relevance of AR loss in advanced prostate cancer. To study this, we queried the recently published genomics dataset and we found that, in fact, AR negative prostate cancer is quite prevalent, accounting for > 10% of cases of CRPC. This finding could potentially impact CRPC management through: 1) the identification of a novel resistance mechanism (loss of AR) that can be detected in clinical specimens, 2) the identification of novel signaling pathways that can drive AR negative prostate cancer, and 3) enabling us to understand how SGK1 can lead to cellular lineage transdifferentiation.

Our identification of a subset of prostate cancer patients with loss of AR enabled us to identify novel biological pathways, which might represent candidate therapeutic targets for AR negative CRPC. We hypothesize that these biological pathways might also account for patients AR positive disease who have sustained AR-directed therapy-dependent inhibition of AR activity.

OTHER DISCIPLINES

1. Using CRISPR to generate isoform specific gene knockouts

Protein variants of common oncogenes have received increasing attention in the cancer biology community. Examples of this include the role of AR-v7 in driving resistance to AR-targeted therapy as well as the isoforms of ALK oncogene (8) (9). Our finding that translational isoforms of GR can drive resistance to enzalutamide led us to explore novel ways of engineering isoform-specific cellular models in the laboratory. We were able to leverage CRISPR in a novel way so as to introduce frameshift alterations in the DNA sequence downstream of the full-length isoform and upstream of the truncated isoform translational start site. This enabled deletion of the full-length isoform and retention of the truncated isoform. This method will enable the study of translational isoform variants of any gene of interest and will impact the broader cell biology and cancer biology community.

TECHNOLOGY TRANSFER: nothing to report

SOCIETY: nothing to report

CHANGES/PROBLEMS
Nothing to report.

PRODUCTS
Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
Project Participants
Name: David Wise
Project Role: Project PI
Researcher Identifier: N/A
Nearest person month worked: 7
Contribution to Project: David Wise planned all aspects of this project and contributed to the experimental procedures.

Name: Sarala Kal
Project Role: Laboratory Technician
Researcher Identifier: N/A
Nearest person month worked: 12
Contribution to Project: Sarala Kal performed tissue culture, cell line engineering, and biochemical techniques, including immunoblotting and qRT-PCR.
Funding Support: 5R01CA193837-02 (PI: Charles Sawyers)

Changes in Support for Key Personnel
Charles Sawyers – yes (report to be found at the end of this document)
David Wise – no changes to report
Other partner organizations: No changes to report

SPECIAL REPORTING REQUIREMENTS: None

APPENDICES: None

REFERENCES

1. Gao, D., Vela, I., Sboner, A., Iaquinta, Phillip J., Karthaus, Wouter R., Gopalan, A., Dowling, C., Wanjala, Jackline N., Undvall, Eva A., Arora, Vivek K., Wongvipat, J., Kossai, M., Ramazanoglu, S., Barboza, Luendreo P., Di, W., Cao, Z., Zhang, Qi F., Sirota, I., Ran, L., MacDonald, Theresa Y., Beltran, H., Mosquera, J.-M., Touijer, Karim A., Scardino, Peter T., Laudone, Vincent P., Curtis, Kristen R., Rathkopf, Dana E., Morris, Michael J., Danila, Daniel C., Slovin, Susan F., Solomon, Stephen B., Eastham, James A., Chi, P., Carver, B., Rubin, Mark A., Scher, Howard I., Clevers, H., Sawyers, Charles L., and Chen, Y. (2014) Organoid Cultures Derived from Patients with Advanced Prostate Cancer. *Cell* **159**, 176-187
2. Lu, N. Z., and Cidlowski, J. A. (2005) Translational Regulatory Mechanisms Generate N-Terminal Glucocorticoid Receptor Isoforms with Unique Transcriptional Target Genes. *Molecular Cell* **18**, 331-342
3. Arora, Vivek K., Schenkein, E., Murali, R., Subudhi, Sumit K., Wongvipat, J., Balbas, Minna D., Shah, N., Cai, L., Efstathiou, E., Logothetis, C., Zheng, D., and Sawyers, Charles L. (2013) Glucocorticoid Receptor Confers Resistance to Antiandrogens by Bypassing Androgen Receptor Blockade. *Cell* **155**, 1309-1322
4. Robinson, D., Van Allen, Eliezer M., Wu, Y.-M., Schultz, N., Lonigro, Robert J., Mosquera, J.-M., Montgomery, B., Taplin, M.-E., Pritchard, Colin C., Attard, G., Beltran, H., Abida, W., Bradley, Robert K., Vinson, J., Cao, X., Vats, P., Kunju, Lakshmi P., Hussain, M., Feng, Felix Y., Tomlins, Scott A., Cooney, Kathleen A., Smith, David C., Brennan, C., Siddiqui, J., Mehra, R., Chen, Y., Rathkopf, Dana E., Morris, Michael J., Solomon, Stephen B., Durack, Jeremy C., Reuter, Victor E., Gopalan, A., Gao, J., Loda, M., Lis, Rosina T., Bowden, M., Balk, Stephen P., Gaviola, G., Sougnez, C., Gupta, M., Yu, Evan Y., Mostaghel, Elahe A., Cheng, Heather H., Mulcahy, H., True, Lawrence D., Plymate, Stephen R., Dvinge, H., Ferraldeschi, R., Flohr, P., Miranda, S., Zafeiriou, Z., Tunariu, N., Mateo, J., Perez-Lopez, R., Demichelis, F., Robinson, Brian D., Schiffman, M., Nanus, David M., Tagawa, Scott T., Sigaras, A., Eng, Kenneth W., Elemento, O., Sboner, A., Heath, Elisabeth I., Scher, Howard I., Pienta, Kenneth J., Kantoff, P., de Bono, Johann S., Rubin, Mark A., Nelson, Peter S., Garraway, Levi A., Sawyers, Charles L., and Chinnaiyan, Arul M. (2015) Integrative Clinical Genomics of Advanced Prostate Cancer. *Cell* **161**, 1215-1228
5. Tan, H.-L., Sood, A., Rahimi, H. A., Wang, W., Gupta, N., Hicks, J., Mosier, S., Gocke, C. D., Epstein, J. I., Netto, G. J., Liu, W., Isaacs, W. B., De Marzo, A. M., and Lotan, T. L. (2014) Rb Loss is Characteristic of Prostatic Small Cell Neuroendocrine Carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* **20**, 890-903
6. Chen, H., Sun, Y., Wu, C., Magyar, C. E., Li, X., Cheng, L., Yao, J. L., Shen, S., Osunkoya, A. O., Liang, C., and Huang, J. (2012) Pathogenesis of prostatic small cell carcinoma involves the inactivation of the P53 pathway. *Endocrine-related cancer* **19**, 321-331

7. Tzelepi, V., Zhang, J., Lu, J.-F., Kleb, B., Wu, G., Wan, X., Hoang, A., Efstathiou, E., Sircar, K., Navone, N. M., Troncoso, P., Liang, S., Logothetis, C. J., Maity, S. N., and Aparicio, A. M. (2012) Modeling a Lethal Prostate Cancer Variant with Small-Cell Carcinoma Features. *Clinical Cancer Research* **18**, 666
8. Antonarakis, E. S., Lu, C., Wang, H., Luber, B., Nakazawa, M., Roeser, J. C., Chen, Y., Mohammad, T. A., Chen, Y., Fedor, H. L., Lotan, T. L., Zheng, Q., De Marzo, A. M., Isaacs, J. T., Isaacs, W. B., Nadal, R., Paller, C. J., Denmeade, S. R., Carducci, M. A., Eisenberger, M. A., and Luo, J. (2014) AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer. *New England Journal of Medicine* **371**, 1028-1038
9. Wiesner, T., Lee, W., Obenauf, A. C., Ran, L., Murali, R., Zhang, Q. F., Wong, E. W. P., Hu, W., Scott, S. N., Shah, R. H., Landa, I., Button, J., Lailler, N., Sboner, A., Gao, D., Murphy, D. A., Cao, Z., Shukla, S., Hollmann, T. J., Wang, L., Borsu, L., Merghoub, T., Schwartz, G. K., Postow, M. A., Ariyan, C. E., Fagin, J. A., Zheng, D., Ladanyi, M., Busam, K. J., Berger, M. F., Chen, Y., and Chi, P. (2015) Alternative transcription initiation leads to expression of a novel ALK isoform in cancer. *Nature* **526**, 453-457

OTHER SUPPORT
Sawyers, Charles L.

CONTIUING ACTIVE:

Howard Hughes Medical Institute (PI: Sawyers) 3/1/08-
Major Goals of this project: Patient oriented research into molecularly targeted therapy of cancer
Agency Contact: Edit Biro, MAS, Howard Hughes Medical Institute, 1230 York Ave, Box 269, New York, NY 10065
Overlap: None

5 R01 CA155169-05 (PI: Sawyers) 5/1/12-3/31/17 2.4 calendar
NCI/NIH $235,305 ($166,000 to lab)
Understanding Resistance to Next Generation Antiandrogens
Specific Aims: To explore the molecular basis by which GR selectively activates certain AR target genes (Aim 1), the functional role of GR, AR and the GR/AR target gene SGK1 in maintaining drug resistance (Aim 2), and the clinical relevance of these findings in circulating tumor cells obtained from patients at treatment start and at relapse (Aim 3).
Agency Contact: Grants Management Specialist: Renee Carruthers, carruthersr@mail.nih.gov, Phone: 301-496-9310, Fax: 301-451-5391
Overlap: None

1 R01 CA19387-02 (PI: Sawyers) 4/1/2015 -3/31/2020 2.40 calendar
NCI $ 289,054 ($231,878 to lab)
Defining the Role of ERG in Modulating the AR Cistrome and Antiandrogen Sensitivity
Specific Aims: This project will shed light on the molecular mechanism by which ERG causes prostate cancer and the impact of ERG on response to therapies directed against the androgen receptor, the common form of treatment for metastatic prostate cancer.
Aim 1. Decipher the mechanism of ERG-mediated reprogramming of the AR cistrome
Aim 2. Understand the role of PTEN loss in modulating the ERG transcriptome.
Aim 3. Determine the role of ERG expression in sensitivity to AR and PI3K inhibition.
Agency Contact: Funmi Elesinmogun, Email: elesinmf@mail.nih.gov, Phone: (240) 276-6313
Overlap: None

5 P50 CA092629-15 (PI:Scher) 9/14/01 – 8/31/16 2.4 calendar
National Institutes of Health $ 95,102
MSKCC Spore in Prostate Cancer
Major Goals of this Project: This allocation is split between three projects: Project 1, Project 6 and Core C. Project 1 covers a campus-wide oncogenome project, Project 6 serves to enhance the clinical development of a novel antiandrogen named A52 and the Core covers animal maintenance and experimentation costs.
Role: Co-Leader of Project 1 and 4, Co-Leader of Mouse Core D and Admin Core F
Specific Aims:
1. Conduct a large-scale evaluation of promising molecular biomarkers that are potentially useful for outcome prediction using routinely processed prostate cancer tissue samples from both conservatively treated British watchful waiting patients and aggressively treated MSKCC patients (Project 1).
2. Develop an outcome prediction algorithm for early-stage prostate cancer based on comprehensive molecular analysis of
a large series of well-annotated prostate cancer samples with clinically significant endpoints (Project 1).
3. Develop robust assays for determination of the predictive signature in the clinical setting (Project 1).
4. Independently test the final predictive algorithm in a wide spectrum of patients with prostate cancer to evaluate predictive accuracy and establish the general utility of the clinical test (Project 1).
5. Conduct a Phase 1 Clinical Trial of A52 in Men with castrate-resistant prostate cancer (Project 6).
6. To Evaluate the Activity of MDV3100 and A52 in Combination with Abiraterone in Preclinical Models of CRPC (Project 6).
7. Material support for P01 research projects (Mouse Core C).
Agency Contact: Renee Carruthers, Email: carruthersr@mail.nih.gov Phone: 301-631-3018 Fax: 301-451-5391
Overlap: None

5 P30 CA008748-50 (PI: Thompson) 1/1/14-12/31/18 1.80 calendar
NCI $0 to lab
Cancer Center Support Grant (Cancer Biology and Experimental Pathology Program)
The CCSG funds support MSK's research infrastructure. These shared resources facilitate the research activities of the clinical, translational and laboratory programs at the Cancer Center.
Agency Contact: Funmi Elesinmogun, Email: elesinmf@mail.nih.gov, Phone: (240) 276-6313
Overlap: None

2 T32 CA160001-06 (PI: Sawyers) 7/11/2011 - 7/31/2021 0.00 calendar
NCI $239,944 ($0 to lab)
Translational Research in Oncology Training Program
The training program for translational cancer research will provide opportunities to postdoctoral PhD trainees to learn about human oncology and pathogenesis, and work collaboratively with clinicians to advance the treatment of cancer patients. The goals are: to help basic scientists to develop a strong clinical background so that they may effectively bring discoveries from bench to bedside; and to foster interdisciplinary research and collaboration.
Agency Contact: Renee Carruthers, Email: carruthersr@mail.nih.gov, Phone: 301-631-3018, Fax: 301-451-5391
Overlap: None

NEW ACTIVE:

W81XWH-15-1-0274 (PI: Mu) 8/1/2015 - 7/31/2017 0.12 calendar
CDMRP $54,960
Identifying biomarkers of antiandrogen resistance: An shRNA-based in vivo screening approach
Specific Aims: To investigate the underlying mechanisms of enzalutamide resistance caused by disruption of RB1 and TP53 pathways and determine potential therapeutic targets and clinical relevance. **(2)** To perform an shRNA-based *in vivo* screen of the prostate cancer deletome to identify the biomarker genes that confer resistance to enzalutamide.
Agency Contact: Janet P Kuhns, Email: janet.p.kuhns.civ@mail.mil, Phone: 301-619-2827
Overlap: None
*****Dr. Sawyers is a mentor for this grant

W81XWH-15-1-0276 (PI: Wise) (THIS GRANT) 8/1/2015 - 7/31/2017 0.12 calendar
CDMRP $57,500
Identifying androgen receptor-indepdent mechanisms of prostate cancer resistance to second generation anti-androgen therapy
Specific Aims: 1. Modulate SGK1 expression in a previously established GR-driven prostate cancer model and determine the effects on resistance to enzalutamide and AR activity
2. Evaluate the importance of EMT-related cellular reprogramming in SGK1-dependent enzalutamide resistance.
3. Determine the importance of SGK1-dependent cellular reprogramming in a 3D organoid human prostate cancer culture system.
Agency Contact: Janet P Kuhns, Email: janet.p.kuhns.civ@mail.mil, Phone: 301-619-2827
Overlap: None
*****Dr. Sawyers is a mentor for this grant

COMPLETED:

Stand up to Cancer Dream Team Award (PI: Chinnaiyan) 8/1/12-7/31/15 1.2 calendar*
SU2C/AACR $335,390/yr direct to MSK
Precision Therapy of Advanced Prostate Cancer

Project Goals/Aims: The goal of this project is to establish a precision medicine paradigm for castration-resistant prostate cancer. This proposal primarily funds the collection of clinical trial biopsies, sequencing and computational analysis.
Aim 1: Establish a multi-institutional infrastructure incorporating 5 leading prostate cancer clinical sites,

2 sequencing and computational analysis sites, linked with appropriate sample and data coordination.
Aim 2: Establish a prospective cohort of 500 patients (the ―CRPC 500‖) utilizing the multi-institutional infrastructure to support the clinical use of integrative prostate cancer sequencing, analysis, and clinical trial decision making.
Aim 3: Conduct parallel, preclinical in vivo functional studies of resistance biomarkers and of SU2CPCF sponsored combination therapies.
Aim 4: Identify molecular determinants of abiraterone sensitivity and acquired resistance in patients.
Aim 5: Conduct clinical trials of novel combinations targeting AR and/or the PTEN pathway, based on existing preclinical data and an understanding of resistance mechanisms.
Aim 6: Identify molecular determinants of sensitivity and acquired resistance to PARP inhibitors in patients. **Agency Contact:** Margaret Foti, MD, PhD, Stand up to Cancer, c/o AACR, 615 Chestnut Street, 17th Floor Philadelphia, PA 19106

3 P30 CA008748-49 S2 (PI: Thompson) 1/1/15-12/31/15 0.12 calendar
NCI $133,333 ($0 to lab)
Cancer Center Support Grant
We hypothesize that lineage-specific requirements for prostate epithelial cell growth, shared between malignant and benign underlie the unique difficulty in propagation of prostate cancer cells.
Agency Contact: Funmi Elesinmogun, Email: elesinmf@mail.nih.gov, Phone: (240) 276-6316
Overlap: None

GC220267 (PI: Sawyers) 1/1/2013 - 12/31/2015 0.12 calendar
Prostate Cancer Foundation $ 250,000
Prostate Organoid Proposal
Prostate organoid technology has the potential to revolutionize the prostate cancer field by:
(1) generating a tissue bank of several hundred matched tumor/normal human organoid pairs from localized cancers that represent the genomic diversity of primary prostate cancer
(2) establishing a tissue bank of metastatic prostate cancer organoids
Agency Contact: Howard Soule, Chief Scientific Officer, www.pcf.org, Phone: 310-570-4596
Overlap: None